Powdered calcium carbide in 99.63 alcohol yielded visible gas bubbles[9] after a few seconds, while $KmnO_4$ crystals showed no color after five minutes.

SUMMARY

(1) The above is recommended as an economical, convenient and quick method for producing absolute alcohol on a laboratory scale.

If the distillation is executed with free flame, excessive or careless heating must be avoided near the end of the operation because of the copper acetylide in the residue.

(2) Calcium carbide is recommended over potassium permanganate or anhydrous copper sulphate as a qualitative reagent in detecting traces of water in alcohol.

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SPECIAL ARTICLES

MANOILOV'S REACTION FOR IDENTIFICATION OF THE SEXES[1]

IN a recent number of the *Russian Bulletin for Applied Botany and Plantbreeding* two papers[2] were published dealing with a problem which may have a great influence on the further development of studies on the physiology of sex. Since these papers deal with a subject in which many biologists are interested and since they are published in a periodical and language not very accessible to most of them it seems to us justifiable to give a short review of those papers and at the same time to present our tests of Dr. Manoilov's method.

Dr. Manoilov found a reaction[3] by which it is possible to distinguish female blood from male. The following reagents were used:

(1) 1 per cent. solution of Papayotin in water.
(2) 1 per cent. solution of Dahlia or Grübler's methyl-green in alcohol.
(3) 1 per cent. solution of potassium permanganate in water.
(4) 40 per cent. solution of HCl.
(5) 2 per cent. solution of thiosinamin in water.

To 3 cc of 10 to 20 per cent. blood 10 drops of the first solution were added, after one or two minutes 3 drops of the second solution, then 10 drops of the third, 1 to 3 drops of the fourth and 5 drops of the fifth solution were added. After additions of the first, second and third solutions the material has to be stirred (not shaken), and after the addition of the fourth and fifth reagents it has to be shaken. The male blood soon becomes colorless or nearly so, while female blood retains its reddish-violet color.

Dr. Manoilov is inclined to believe that hemoglobin is responsible for the differential male and female reactions he obtained, and since chlorophyll is chemically closely related to hemoglobin he tried to see if the same reaction would occur in dioecious plants. An alcoholic extract of chlorophyll from leaves was made by letting 10g of leaves stand for two hours in 30 cc of 60 per cent. alcohol. To 3 cc of that extract the same reagents were added in the way described above. Positive results were obtained, *e.g.*, solutions from male plants became colorless and those from female plants retained the color. Experiments were made with Acer negundo, Lychnis dioica, Vallisneria, Urtica and Cannabis, all giving the same results.

Grünberg tested Manoilov's method on the following plants: Vallisneria sp, Urtica dioica, Cannabis sativa, Populus sp, Hippophae rhamnoides, Eucephialartos villosus, E. longifolius and Begonia sp. Especially interesting results were obtained with monoecious Begonia: male flowers gave the characteristic male reactions, female flowers gave the female reaction and the leaves an intermediate one. The experiments of Grünberg showed that the presence of chlorophyll is not essential for the reaction to occur. This was confirmed by the results of investigations conducted by Minenkov[4] who obtained differential sexual reaction using seed and young seedlings of dioecious plants. Minenkov's method, however, was entirely different from that used by Manoilov.

Experiments to test Manoilov's method are being made by the authors, and positive results have been obtained with the following organisms:

[9] The calcium carbide used in a qualitative test should be freed from occluded air, as this, when alcohol is added, will give air bubbles which may be mistaken for acetylene gas bubbles. The discharge of occluded air will cease within a few seconds; the generation of acetylene, if water is present, will continue for a time.

[1] Research carried on with the aid of a grant from the committee for research in problems of sex of the National Research Council.

[2] Manoilov, E. O., "Identification of the sexes in dioecious plants by chemical reaction." *Bul. Appl. Bot. and Plantbreed.* 13(2): 503–505. 1922–1923.

Grünberg, O., An addition to the paper of Dr. Manoilov: "Identification of the sexes in dioecious plants by chemical reaction." *Bul. Appl. Bot. and Plantbreed.* 13(2): 506. 1922–1923 (Both in Russian).

[3] Manoilov's paper describing the method which he used in experiments with animals was published in *Vratschebnaja Gazeta* (*Medical Gazette*) No. 15, 1923. Unfortunately, it has not been possible for us as yet to obtain this paper.

[4] Minenkov, A., *Nautschno Agronomitscheskii Zurnal* (*Jour. Sci. Agr.*), Vol. 1, 1924.

	Part of the organism used	Number of individuals tested ♀	♂
Mice	blood	12	9
Sheep	blood	2	2
Pigeon	blood serum	3	3
Fruit flies (Drosophila virilis)	.5–1.5 g. of flies	2	2
Cladocera sp.	.2 g. of animals	3	2
Salix sp.	catkins	5	7
Populus sp.	catkins	2	6

It may be noted that the reaction is very sensitive. A slight mistake in the method or fault in the material may give inconclusive results. It should be mentioned that nine more male mice than these previously listed were used in investigations. Six of them gave inconclusive results and with the other three a reaction was obtained which was classified as female. It is very likely that in those cases, due to our unfamiliarity with technic, reacting enzymes were destroyed and the expected reaction failed to occur.

As pointed out by Manoilov in making the chlorophyll extract, it is essential not to have alcohol stronger than 60 per cent. and not to leave the material in alcohol over 24 hours. Otherwise the enzymes which produce the reaction are likely to be destroyed.

The authors are indebted to Dr. C. B. Davenport for mice and sheep material, to Dr. A. M. Banta for Cladoceras and to Dr. O. Riddle for pigeon material.

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HYDROGEN-MAGNESIUM HALIDES[1]

THE intermediate formation of hydrogen-magnesium halides is postulated in current theories to account for the reducing action of the Grignard reagent on the carbonyl linkage. Several attempts have been made to prepare such compounds: first, the action of hydrogen halides on magnesium at low temperatures; second, the heating of sec-butylmagnesium bromide at 150° in a high vacuum, and, third, the catalytic reduction of RMgX compounds. Catalytic reduction of triphenylmethyl-magnesium chloride gave results which are indicative of the formation of hydrogen-magnesium chloride.

The reaction of RMgX compounds with azo compounds and the formation of colored intermediate compounds when the Grignard reagent reacts with carbonyl compounds suggests the intermediate formation of magnesium halides analogous to the metal ketyls. The free radical (R) so formed then dissociates to give hydrogen and the corresponding unsaturated compound. This hydrogen then combines with the trivalent carbon.

RUSSELL M. PICKENS

[1] Abstract of a paper presented before the meeting of the American Chemical Society at Baltimore.

THE AMERICAN CHEMICAL SOCIETY[1]

The use of the sodium salt of dimethylglyoxime for the qualitative and quantitative determination of nickel: WALDO L. SEMON and GILBERT SWART. Dimethylglyoxime forms a crystalline hydrated sodium salt. It is extremely soluble in water and insoluble in alcohol. A 3 per cent. (0.1M) *aqueous* solution is recommended for use in qualitative and quantitative analysis to replace the 1 per cent. alcoholic solution of dimethylglyoxime. Analyses for Ni are given in the presence of Co, Cr, Mn, Zn and Fe and in various alloy steels.

Esters of 3-amino-4-hydroxymethyl-benzoic acid: FRANCIS H. CASE. Ethyl 3-amino-4-hydroxymethyl-benzoate is prepared as follows: p-cyanobenzyl chloride is nitrated, and the 3-nitro-4-chloromethyl-benzonitrile converted to the acetate. This compound on hydrolysis with alcoholic hydrogen chloride yields ethyl 3-nitro-4-hydroxymethyl-benzoate. The latter is reduced to the amino ester by tin and alcoholic hydrogen chloride. Better yields were obtained with colloidal platinum in acetic acid solution. The butyl amino ester is similarly prepared from the butyl nitro ester. The hydrochlorides are soluble in water. The local anesthetic action of these esters will be investigated. A simple method of preparing p-chloromethyl benzoic acid has been found, in which the corresponding nitrile is hydrolyzed with conc. HCl, and the acid recrystallized from alcohol; yield, 80 per cent. On attempted recrystallization from water, p-hydroxymethyl benzoic acid results. Under the same hydrolytic conditions p-cyanobenzyl bromide is also converted into p-chloromethyl benzoic acid.

The reactivity of the hydroxyl group in certain alcohols: JAMES F. NORRIS and HENRY D. HIRSCH. The reactivity of the hydroxyl group in nine straight chain aliphatic alcohols was measured by determinations of their rates of reaction with aqueous hydrobromic acid at 100°. The rates were found to be related in a quantitative manner to the structure of the alcohols. If a

[1] The Los Angeles Meeting, August, 1925, of the Divisions of Organic Chemistry and of Medicinal Products.